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Exhibit A

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Title

Crystallization and structure determination of Staphylococcus aureus NAD synthetase

Data entry by : JONES, KIMBERLY **Team :** 2800 **Date:** 04/30/2001

Research; Laguna Niguel, CA), Wizard I, Wizard II, Cryo I, Cryo II (Emerald Biostructures; Bainbridge Island, WA). The protein was screened with the hanging drop method using 0.5 mL volume of the crystallization agent in the well and 1μL + 1μL protein drops. A hit (crystal) was observed in Crystal Screen 1-Lite (Hampton Research), condition 43: 15% PEG 1500. Although there is no buffer in this condition, the pH of the drop was approximately 7.5 as measured by a pH microprobe. The crystals were thin plates with a length of 0.4-0.7mm (Fig. 3). Crystals were observed three days after setup. Crush tests suggested the crystals were composed of protein due to the powdering effect observed when touching the crystal with a metal probe. Izit dye (Hampton Research) successfully stained the crystals blue, confirming the crystals were protein. Refinement of the condition yielded crystals of monoclinic morphology with a range of dimension of 0.15-0.8 x 0.2 x 0.05-0.1 mm (Fig. 4). Crystals generally grew to full size within 96 hours. Selenomethionine NAD synthetase was also crystallized in the same conditions. Co-crystallizations with $N^{a}AD$ yielded crystals with a tetragonal or pyramidal morphology on the order of 0.4 x 0.2 x 0.05-0.1 mm in size. A series of experiments were undertaken to find solution conditions that would permit freezing of the NAD synthetase crystals at 100K without producing ice crystals. Early experiments led to rapid cracking of the crystals due to variability in the pH of the cryogenic solution compared to the pH of the crystallization drop. Successful conditions were eventually found to be 20% PEG 1500, 15% glycerol, 0.05M Imidazole, pH 7.0. Crystals were soaked in ten minute intervals in solutions containing increasing amounts of glycerol starting at 10% and increasing in 5% increments until 20% glycerol was reached. Crystals were then flash frozen in liquid nitrogen. Crystals diffracted to 3.5Å using laboratory X-rays and 2.2Å using synchrotron radiation. Data collection and structure solution will be the focus of a future study report.

CONCLUSIONS AND ACKNOWLEDGMENTS

The availability of purified NAD synthetase early in the target development cycle has facilitated the crystallization of this target. These crystals and the subsequent structure determination demonstrate the validity of this target for structure directed drug discovery. The rapid progress in these structural biology efforts suggests that once suitable inhibitors for NAD synthetase are obtained, structural chemistry information about the interaction of the inhibitor with the protein should rapidly follow.

Exhibit A